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Quantification of carvedilol in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry Application to bioequivalence study

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Abstract

A rapid, sensitive and specific method to quantify carvedilol in human plasma using metoprolol as the internal standard (IS) is described. The analyte and the IS were extracted from plasma by liquid–liquid extraction using a diethyl-ether solvent. After removed and dried the organic phase, the extracts were reconstituted with a fixed volume of acetonitrile–water (50/50; v/v). The extracts were analyzed by a high performance liquid chromatography coupled to electrospray tandem mass spectrometry (HPLC–MS/MS). Chromatography was performed isocratically on Alltech Prevail C_{18} 5 μm analytical column, (150 mm × 4.6 mm i.d.). The method had a chromatographic run time of 3.5 min and a linear calibration curve over the range 0.1–200 ng ml^{-1} ($r^2 > 0.997992$). The limit of quantification was 0.1 ng ml^{-1}. This HPLC–MS/MS procedure was used to assess the bioequivalence of two carvedilol 25 mg tablet formulations (carvedilol test formulation from Laboratórios Biosintética Ltda and Coreg® from Roche Químicos e Farmacêuticos S.A standard reference formulation). A single 25 mg dose of each formulation was administered to healthy volunteers. The study was conducted using an open, randomized, two-period crossover design with a 2-week wash-out interval. Since the 90% CI for C_{max} and AUCs ratios were all inside the 80–125% interval proposed by the US Food and Drug Administration Agency, it was concluded that carvedilol formulation elaborated by Laboratórios Biosintética Ltda is bioequivalent to Coreg® formulation for both the rate and the extent of absorption.

Keywords: Healthy volunteer; Plasma; Pharmacokinetics; Carvedilol; LC–MS/MS; Bioequivalence

1. Introduction

Carvedilol is a nonselective β-blocking agent [1,2] and it also has vasodilating properties that are attributed mainly to its blocking activity at receptors. Carvedilol is a racemic compound and the nonselective β-blocking activity resides mainly in the (*S*)-carvedilol, while the α-blocking activity is shared by (*R*)- and (*S*)-enantiomers [3,4], but this drug is used clinically as a racemic mixture of both enantiomers. Carvedilol is used in the treatment of mild to moderate hypertension and angina pectoris [5] and is often used in combination with other drugs. Carvedilol is a anti-hypertensive agent with non-selective β- and α1-adrenergic receptor blocking activities [6] which is also being used in the treatment of congestive heart failure [7,8] and presents antioxidative effects

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in vivo [9]. Carvedilol has been determined in plasma and other biological fluids such as high performance liquid chromatography coupled to fluorometric detection [6,10–18], high performance liquid chromatography coupled to ultraviolet detection [18], capillary electrophoresis coupled to ultra-violet detection [18,19], capillary electrophoresis coupled with laser-induced fluorescence [20], high performance liquid chromatography coupled to electrochemical detection [21], liquid chromatography coupled to tandem mass spectrometry [22,23]. Carvedilol is rapidly and completely absorbed after oral administration, but its absolute bioavailability is rather low due to an extensive first-pass metabolism [24].

Here we present a fast, sensitive and selective method for measuring plasma carvedilol using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) with positive ion electrospray ionization using multiple reaction monitoring (MRM) mode to quantify carvedilol in human plasma using metoprolol as the internal standard (IS, Fig. 1). This method was employed in a bioequivalence study of two carvedilol 25 mg tablet formulations: carvedilol test formulation from Laboratórios Biosintética Ltda and Coreg® from Roche Químicos e Farmacêuticos S.A standard reference formulation. The bioequivalence study was conducted using a single dose, two-way, open, randomized crossover design with 2-week wash-out period between the doses and 36 healthy volunteers were included.

2. Experimental

2.1. Chemicals and reagents

Carvedilol (99.6%) was provided by Heatwell S.A. Metoprolol (100.2%) was obtained from, Novartis, respectively. Acetonitrile, methanol (HPLC-grade) and ammonium acetate, analytical grade, were purchased from J. T. Baker (Phillipsburg, NJ, USA), diethyl ether (analysis grade) was purchased from Mallinckrodt (Paris, KY, USA), deonized water (analysis grade) was purchased from Millipore (Brazil) and formic acid (86%, analytical- grade) was purchased from Cetus (Brazil). Ultra pure water was obtained from an Elga UHQ system (Elga, UK). Blank human blood was collected from healthy drug-free volunteers. Plasma was obtained by centrifugation of blood treated with the anticoagulant sodium heparin. Pooled plasma was prepared and store at approximately −20 °C until needed.

2.2. Calibration standards and quality control

Stock solutions of carvedilol were prepared methanol–water (70:30, v/v) and internal standard (metoprolol) were prepared in methanol–water (50:50, v/v) at concentrations of 1 mg/ml. Calibration curves of carvedilol were prepared by spiking blank plasma at concentration of 0.1, 0.2, 0.5, 2.0, 10.0, 20.0, 50.0, 100.0 and 200.0 ng/ml. The

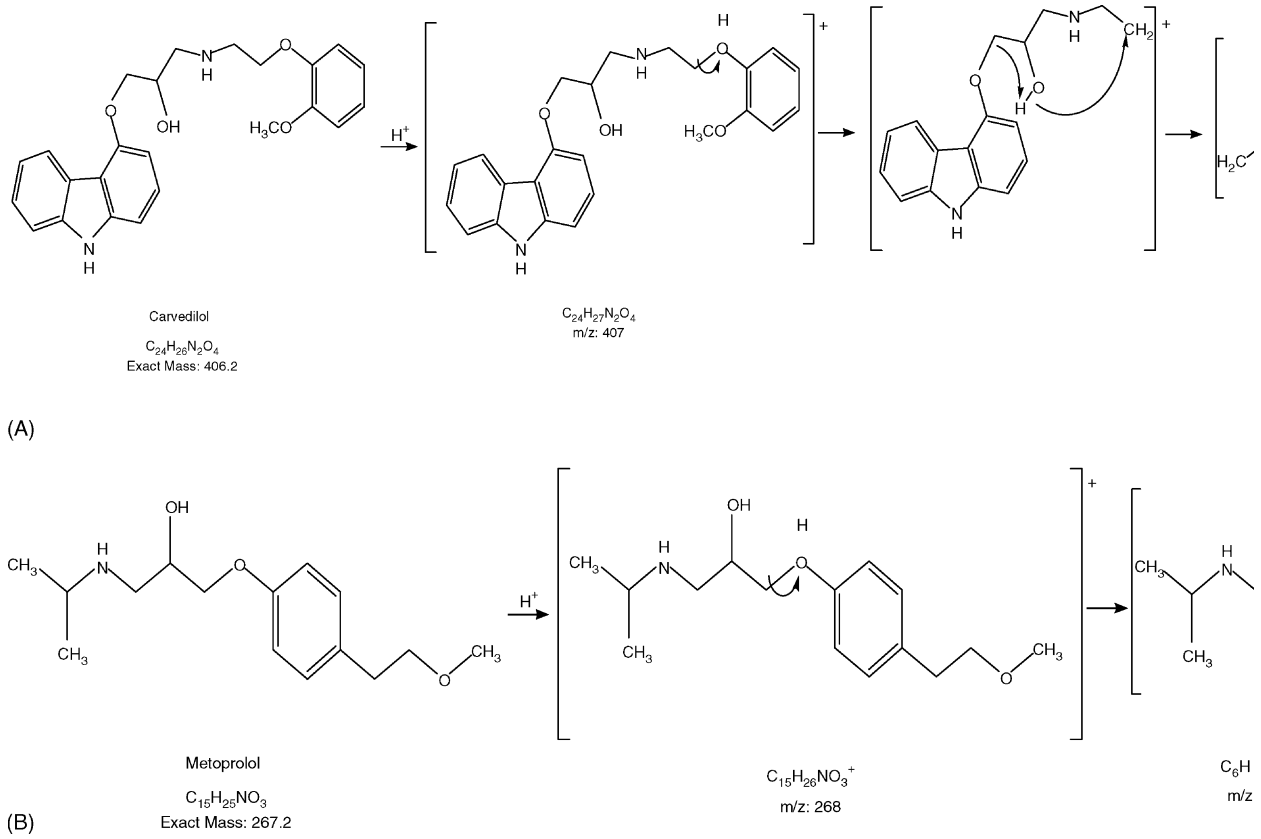

Fig. 1. Proposed fragmentation pathways for the Carvedilol (A) and Metoprolol (B).